PRELIMINARY WATER QUALITY TESTING OF LAKE HURON SHORELINE MUCK SAMPLES

Submitted to Mr. Jim Roland

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Prepared by

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Introduction:

Due to concerns regarding the appearance of a mat of solids plus an algal mixture, washing up on the shoreline of Lake Huron, samples were collected for the assessment of microorganisms associated with fecal contamination. Samples were collected by Mr. Jim Roland at the west and east sides of a dock, which is owned by Mr. Max Tomin (3140 Shore Dr, Port Austin MI. 48467), at the Lake Huron, Huron County, Michigan, and were delivered to the Water Quality and Health Laboratory at Michigan State University on September 6, 2006. The samples underwent 1) microscopic analysis and 2) microbial analysis.

1: MICROSCOPIC ANALYSIS

Samples were supplied directly to Dr. Orlando Sarnelle, Department of Fisheries and Wildlife, Michigan State University. The samples appeared to be the consistency of mud, with decomposed plants, or green algae, which were not possible to identify by a visual inspection. Thus, microscopic analysis was undertaken. Benthic organisms, which are bottom-dwelling organisms without backbones that live in the bottom of the lake, were identified (Figure 1). Benthic diatoms were the most abundant organisms and there were some filamentous green algae, a few filamentous cyanobacteria (fairly rare). A lot of very diverse protozoans were also observed but they were not planktonic. Based on the microscopic analysis, the samples were identified as muck sediments, which were mixture of sediment, detritus and benthic algae.

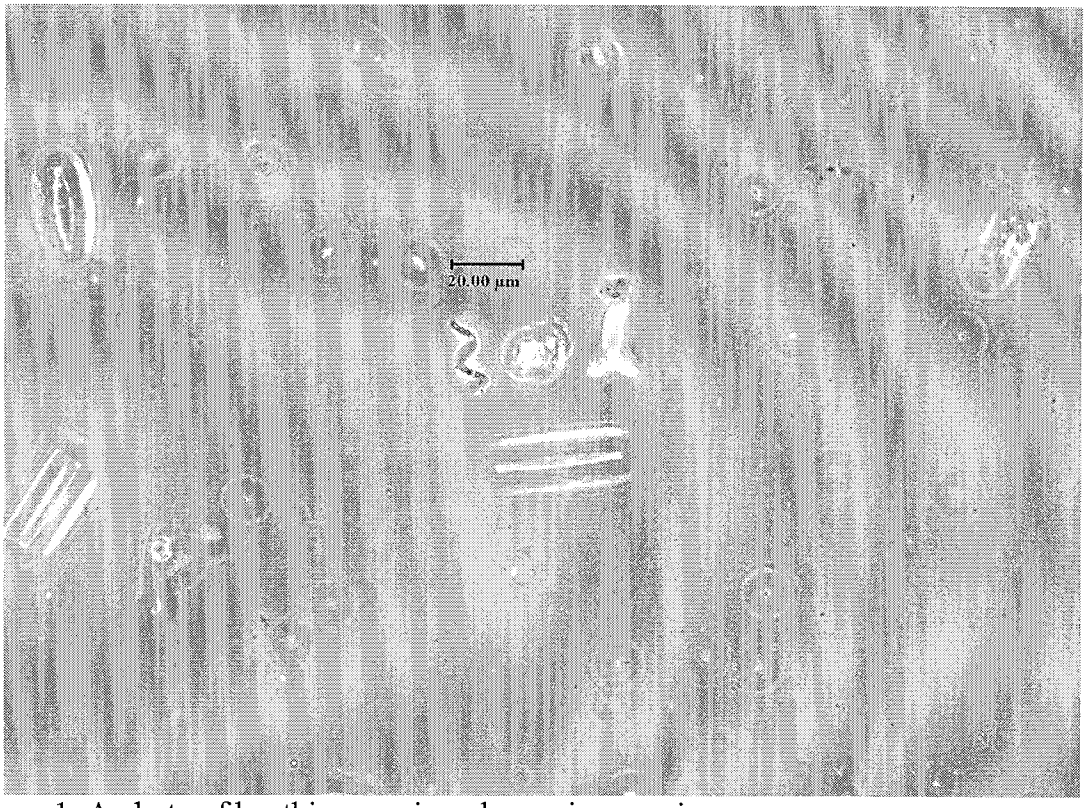

Figure 1: A photo of benthic organisms by a microscopic camera

Muck sediments can be formed as a result of eutrophication by an introduction of excess levels of nutrients and organic matter to the water body (Avenlmelech 1984; FDEP 2002). Runoff, which contains fertilizers, grass clippings, leaves, animal waste, septic waste, etc. may contribute to the production of muck sediments. Some pollutants in the runoff can be transformed within the muck, while the other are decomposed through chemical and microbial process involved in sediment diagenesis (CWP 1994). Other studies have also shown that runoff was significantly associated with recruitment of fecal indicator bacteria to sediments (Cahoon et al. 2006). Thus, the muck sediment samples were evaluated by microbial analysis described in the following section.

2: MICROBIAL ANALYSIS

The muck sediments samples were analyzed for three fecal indicator bacteria; i.e. *Escherichia coli* (*E. coli*), enterococci, *Clostridium perfringens* (*C. perfringens*). These microbes are commonly used by regulatory agencies to evaluate water quality for the presence of waste water or pathogens, typically found in elevated concentrations in human and animal wastes and methods have been standardized for these microorganisms (U.S. EPA 1986). Additionally, the samples were analyzed for coliphage, which is a virus that infects *E. coli*. Coliphage has been suggested as supplementary fecal indicator because coliphage attenuate quickly in environments and are particularly useful for indicating human enteric virus contamination (Havelaar et al., 1993; Payment and Franco, 1993). The detail microbe analysis is given in the appendix. The concentrations of these microbes are expressed in most probable number (MPN), colony formed unit (CFU), and plaque forming unit (PFU) per 100 g of dry muck.

All of the fecal indicator microorganisms were detected from the muck sediment samples at high concentrations ranging from a low of 465 bacterial colony forming units (CFU) of Enterococci and *Clostridium* per 100g to as high as 13, 300 CFU/ 100 g of *E.coli* (Table 1). The results demonstrate that the water body has been receiving fecal inputs in which the solids have likely been deposited to the sediments. The concentrations of microbes at the muck collected at the east side were slightly more contaminated than at the west side. However, the statistical significance between the pollution at the east side of the dock compared to the west could not be determined due to a limited sample analysis.

Table 1: Microbial indicators in the muck sediments

Microbial Indicators	at the west side	at the east side
E. coli (MPN/100 g dry muck)	3,750	13,300
Enterococci (MPN/100 g dry muck)	465	2,610
Enterococci (CFU/100 g dry muck)	2,560	5,180
C. perfringens (CFU/100 g dry muck)	465	1,550
Coliphage (PFU/100 g dry muck)	2,330	2,580

It should be noted that *E. coli*, and enterococci can on occasion be found in the environment in the absence of a known sewage or septic tank contamination (Carillo et al. 1985; Wright 1986; Rivera et al. 1988) and can multiply within tropical environments (Wright 1989; Hardina and Fujioka 1991; Roll and Fujioka 1997; Solo-Gabriele et al. 2000; Desmarais et al. 2002). Although Michigan is not tropical environment, enriched

nutrients in the sediments could support survival of the microorganisms of fecal origin (Avnlmelech et al. 1984; Shibata et al. 2004; Cahoon 2006). *C. perfringens*, a spore-forming obligate anaerobe, is not capable of regrowth in aerobic environments but persists for long periods of time even after chlorination. The detection of *C. perfringens* may not be an indicator of recent fecal contamination. The presence of coliphage indicates, however more recent fecal pollution (perhaps within months), as this virus does not regrow in the environment and has a finite life. The over-wintering of the coliphage in Lake Michigan waters is not known..

3. SUMMARY

Based on the microscopic and fecal indicator analyses, the unidentified samples were found to be muck sediments, which have accumulated large concentrations of solids associated with fecal waste contamination. This study did not evaluate how the sediments formed or were released to the surface water from the bottom of the lake but it could be associated with physical processes of lake, e.g. mixing and stratification. Mr. Jim Roland noted that the lake smelled bad during the sampling. The fecal contamination in the Huron Lake Such is defined as nonpoint source (NPS) pollution. NPS pollution is the Nation's leading source of water degradation and it can occur any time activities disturb the land or water (U.S. EPA 2003). Agriculture, forestry, grazing, septic systems, recreational boating, urban runoff, construction, physical changes to stream channels, and habitat degradation are potential sources of NPS pollution (U.S. EPA 2003).

Since NPS pollution may impact human health, it is recommended that further studies to identify actual sources of fecal contamination in the Lake Huron and ensure the lake water is safe for recreational uses be undertaken. Wet weather flows, septic tank tracers and further genetic testing could be undertaken. The testing of the water for viral pathogens to examine risk to swimmers may also be of value.

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Microbial analyses

The moisture contents of the muck sediment samples were from 6 to 12%. Approximately 5 g of wet muck sediments were mixed with 450 mL of a phosphate buffer solution and blended for one minute. This diluted muck solution was used for the microbe analyses.

E. coli and enterococci were analyzed based on a method using a chromogenic substrate (IDEXX, Westbrook, MN). The chromogenic substrate method, or called IDEXX method, utilizes enzymes that are specific to particular microbe groups. These enzymes are attached to dyes, which are then released when the target microbe is present in the sample. Diluted samples were poured into sterile vessels and the reagents; Colilert for *E. coli* and Enterolert for enterococci, were added into the vessel and mixed. The well mixed samples were then poured into the tray (Quanti-Tray/2000, IDEXX, Westbrook, Maine) and the trays were incubated at 35°C for 24 hours for *E. coli* and 41 °C for enterococci analyses. The number of test wells that show the characteristic color or fluorescence under ultra violet (UV) light were then counted and used in conjunction with a standardized table to provide the concentration in terms of the most probable number (MPN).

Enterococci was also analyzed based on the membrane filter (MF) method, which provides a direct count of bacteria based on the development of colonies on the surface of a MF. The MF method involves filtering a given volume of the sample through a 0.45 mm pore size filter membrane (47 mm diameter membrane, Fisher, Pittsburg, PA) that retains the bacteria. The MF method used for enterococci (Method 1600) was based upon the use of a selective medium (mEI agar, Becton Dickinson, Sparks, MD) upon which samples were incubated at 41C° for 24 hours. Colonies with a blue halo were counted as enterococci. *C. perfringens* was analyzed using the MF method (Bisson and Cabeli 1979). The method for *C. perfringens* enumeration was based upon the use of a mCP agar. Samples were incubated anaerobically using an anaerobic chamber fitted with an anaerobic GasPak (BBL GasPak Anaerobic System Envelopes, Becton Dickinson, Sparks,MD) at 44.5 ± 0.5°C for 24 hours. The plates were exposed to ammonium hydroxide fumes after the incubation and dark pink to magenta colonies were counted as *C. perfringens*.

Coliphage was analyzed base on agar overlays (U.S. EPA Method 1601 and 1602). Filtered volumes of the diluted sample were used to enumerate coliphage. *E.coli* C3000 was used as a host. For each sample, 20 ml were syringe-filtered through a 0.45 micron filter. 0.5 ml of host and 2 ml of sample were added to melted top agar before mixing and pouring onto a tryptic soy agar plate (TSA). For each sample, 5 overlays were performed. Overlays were incubated at 37°C for 24 hours, and then assessed for plaque formation (U.S. EPA 2001). All materials and instruments were sterilized prior to the analysis to prevent contamination. A blank sample was analyzed in order to confirm that there was no cross contamination.

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